Formation of propionate and butyrate by the human colonic microbiota

Petra Louis* and Harry J. Flint

Rowett Institute of Nutrition and Health, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK

*Corresponding author; p.louis@abdn.ac.uk; phone +44 1224 438735.

Running title: Propionate and butyrate producing gut microbes

Originality-Significance statement: In recent years there has been a tendency to rely on sequence data to assign function to microorganisms, however, this has at times led to miss-annotations and the wrong conclusions being drawn. This manuscript pulls together the current knowledge on butyrate and propionate metabolism in the human gut by taking account of biochemical studies performed on microorganisms in addition to sequence information. In particular the areas on 1,2-propanediol metabolism and protein metabolism in the gut have, to our knowledge, not been comprehensively reviewed in the context of gut microbiology.

Summary

The human gut microbiota ferments dietary non-digestible carbohydrates into short-chain fatty acids (SCFA). These microbial products are utilized by the host and propionate and butyrate in particular exert a range of health-promoting functions. Here we provide an overview of the metabolic pathways utilized by gut microbes to produce these two SCFA from dietary carbohydrates and from amino acids resulting from protein breakdown. This overview emphasizes the important role played by cross-feeding of intermediary metabolites (in particular lactate, succinate and 1,2-propanediol) between different gut bacteria. The ecophysiology, including growth requirements and responses to environmental factors, of major propionate and butyrate producing bacteria are discussed in relation to dietary modulation of these metabolites. A detailed understanding of SCFA metabolism by the gut microbiota is necessary to underpin effective strategies to optimize SCFA supply to the host.

Introduction

Short chain fatty acids (SCFA) are the major metabolic products of anaerobic fermentation by microbial communities that colonize the mammalian gut, typically reaching total concentrations of 50-200 mM in the human large intestine. They are taken up efficiently by the gut mucosa and have important impacts upon host physiology as sources of energy, as regulators of gene expression and as signaling molecules that are recognized by specific receptors (Morrison & Preston, 2016; Koh *et al.*, 2016). New mechanisms by which SCFA regulate immune cell development and suppress inflammation have been uncovered recently (Louis *et al.*, 2014; Richards *et al.*, 2016). It is apparent however that the three major SCFA, acetate, propionate and butyrate, differ considerably in their potential effects upon host physiology. First, they differ in their fate and tissue distribution, with butyrate being used preferentially as an energy source by the gut mucosa, propionate contributing to gluconeogenesis in the liver and acetate achieving the highest systemic concentrations in blood (Morrison & Preston, 2016). Second, there are differences in interactions with host

proteins (eg. inhibition of histone deacetylases by butyrate and propionate) and receptors (Bolognini *et al.*, 2016). This makes it particularly relevant to consider the microbial origin of these major fermentation products and the potential for changes in diet and gut physiology to affect their relative production rates and concentrations. This brief review will focus on butyrate and propionate as these two acids are most often considered to benefit health, including protection against colorectal cancer in the case of butyrate and promotion of satiety and reduction in cholesterol in the case of propionate (Morrison & Preston, 2016). Acetate is a net fermentation product for most gut anaerobes that is also produced by reductive acetogenesis, and almost invariably achieves the highest concentrations among the SCFA in the gut lumen. In contrast, propionate and butyrate are produced by distinct subsets of gut bacteria. We consider here what is currently known about the phylogenetic distribution of pathways leading to the formation of these two SCFA within the human colonic microbiota and the potential for diverse dietary and environmental factors to differentially modulate their production. Some fermentation products, including lactate, succinate and 1,2-propanediol, do not usually accumulate to high levels in the human colon of healthy adults, as they can also serve as substrates for other bacteria, including propionate and butyrate producers. As the microbial metabolism of these compounds is intricately linked to the degradation of the main dietary substrates, it will be discussed together with propionate and butyrate formation from carbohydrates and proteins, respectively.

Pathways and bacterial groups contributing to butyrate formation from carbohydrates

Butyrate is produced from carbohydrates via glycolysis from the combination of two molecules of acetyl-CoA to form acetoacetyl-CoA, followed by stepwise reduction to butyryl-CoA. Two different pathways are known for the final step in butyrate formation from butyryl-CoA, which proceeds either via butyryl-CoA:acetate CoA-transferase or via phosphotransbutyrylase and butyrate kinase (Louis & Flint, 2009) (Fig. 1). Butyrate-producing species are found interspersed with butyrate non-producing species in the two predominant families of human colonic Firmicutes, *Ruminococcaceae* and *Lachnospiraceae*, as well as in

other Firmicutes families, including *Erysipelotrichaceae* and *Clostridiaceae* (Barcenilla *et al.*, 2000; Louis *et al.*, 2004). We will briefly consider the characteristics of butyrate-producers that belong to the two most abundant families of Firmicutes. We should note that, as summarized in Table 1, many dominant human colonic Firmicutes (eg. *Blautia* spp., *Eubacterium eligens*, *Ruminococcus* spp.) lack the ability to form butyrate from carbohydrates.

Ruminococcaceae. *Faecalibacterium prausnitzii*, one of the most abundant species present in the healthy human microbiota, produces butyrate via butyryl-CoA:acetate CoA-transferase with net consumption of acetate, and acetate stimulates its growth on carbohydrate energy sources (Duncan *et al.*, 2002). While *F. prausnitzii* strains are obligate anaerobes, they also show growth stimulation by low concentrations of oxygen in the presence of riboflavin and reduced compounds such as cysteine or glutathione (Khan *et al.*, 2012). It is hypothesized that this ability may provide a niche for the bacterium to thrive in the proximity of the colonic wall, where oxygen is diffusing in from the bloodstream. Oxygen consumption is accompanied by a decrease in butyrate formation (Khan *et al.*, 2012). *F. prausnitzii* isolates show limited ability to utilize dietary polysaccharides such as starch and hemicellulose for growth, but some strains utilize inulin and pectin derivatives and the ability to utilize uronic acids is widespread (Lopez-Siles *et al.*, 2012). *F. prausnitzii* is depleted in inflammatory bowel disease patients, especially Crohn's disease, and evidence that it has anti-inflammatory action has attracted interest in this species as a potential therapeutic (Quévrain *et al.*, 2016). Similarly *Butyricicoccus pullicaecorum* is also reported to be less abundant in inflammatory bowel disease patients, and might also have therapeutic potential (Eeckhaut *et al.*, 2013). Butyrate production has been reported for other Ruminococcaceae (Table 1), but rather little is known about most of these organisms.

Lachnospiraceae. *Eubacterium rectale* and the closely related *Roseburia* species constitute a major group of butyrate-producing Firmicutes that share the butyryl-CoA:acetate CoA-transferase route for butyrate production and the same genomic organization of their butyrate synthetic genes from acetyl-CoA to butyryl-CoA (Louis & Flint, 2009). In some *Roseburia* strains, particularly at mildly acidic pH, butyrate is almost the sole fermentation acid produced, with net consumption of acetate typically accompanying the formation of butyrate (Kettle *et al.*, 2015). Other strains and species produce formate and lactate in

addition to butyrate (Louis & Flint, 2009). Genome analysis reveals that there is considerable capacity within this group to utilize diet-derived polysaccharides including starch, arabinoxylan and inulin, that varies substantially between strains and species (Sheridan *et al.*, 2016).

Butyrate-producing Lachnopiraceae show considerable divergence in their phylogeny, gene organization and physiology (Louis & Flint, 2009) (Table 1). Other Lachnospiraceae that possess the butyryl-CoA:acetate CoA-transferase gene include *Eubacterium hallii, Anaerostipes hadrus, Coprococcus catus*, un-characterised species related to isolates SS3/4 and M62/1, and some uncultured organisms (Louis *et al.*, 2010; Reichardt *et al.*, 2014). Two species of *Coprococcus*, in common with many *Clostridium* species that belong to other families of Firmicutes, use the butyrate kinase rather than CoA-transferase enzyme for the final step in butyrate formation (Louis *et al.*, 2004; Louis & Flint, 2009). *E. rectale* and *E. hallii* are among the 10 most abundant species reported in the human faecal microbiota (Qin *et al.*, 2010; Walker *et al.*, 2011) (Table 1) and together accounted for 44% of butyryl CoA:acetate CoA-transferase sequences amplified from faecal samples of 10 healthy volunteers (Louis *et al.*, 2010).

Lactate can be produced from carbohydrates by many different gut bacteria (Duncan *et al.*, 2004). *In vitro* incubations of ^{13}C lactate with human intestinal microbiota show that the label is recovered in acetate, propionate and butyrate. The proportions of these products can vary widely, however, with acidic pH favouring butyrate (Belenguer *et al.*, 2007). In addition, there is considerable inter-individual variation in the fate of ^{13}C lactate, which is assumed to reflect differences in the relative abundance of lactate-utilising species within the microbiota (Bourriaud *et al.*, 2005; Morrison *et al.*, 2006). Certain Lachnospiraceae have the ability to grow in the presence of lactate and acetate to produce butyrate, showing an overall net stoichiometry of 4 mols of lactate and 2 mols of acetate producing 3 mols of butyrate (Duncan *et al.*, 2004). These include the abundant species *A. hadrus*, which uses only D-lactate (Allen-Vercoe *et al.*, 2012) and *E. hallii*, which is able to utilize both lactate isomers (Duncan *et al.*, 2004; Muñoz-Tamayo *et al.*, 2011). Lactate oxidation to pyruvate by direct reduction of NAD^+ is energetically unfavourable. Anaerobic lactate utilisers carry a lactate dehydrogenase that operates in complex with an electron-transferring flavoprotein that couples the endergonic NAD^+ reduction to ferredoxin oxidation, in a process called electron confurcation (Weghoff *et al.*, 2014).

Pathways and bacterial groups contributing to propionate formation from carbohydrates

Two pathways are known for the formation of propionate from sugar fermentation by gut bacteria. Most hexose and pentose sugars are processed through the succinate pathway (Fig.2) whereas the deoxy sugars fucose and rhamnose are metabolized by the propanediol pathway (Fig.3).

The succinate pathway is found mainly in Bacteroidetes and in the Negativicutes class of Firmicutes (Reichardt *et al.*, 2014). It is the major route for propionate formation from dietary carbohydrates driven by the abundant Bacteroidetes, and relative Bacteroidetes abundance was found to correlate with relative faecal propionate levels in human volunteers (Salonen *et al.*, 2014). Succinate is a precursor of propionate, but can accumulate in cultures of *Bacteroides* spp. under growth conditions where PEP carboxykinase is repressed, eg. at high pCO_2 and high dilution rates (Caspari & Macy, 1983). Conversion of succinate to propionate also requires vitamin B_{12} and succinate has been shown to accumulate in B_{12}-depleted cultures of *Prevotella ruminicola* (Strobel 1992). Some species of Bacteroidetes, notably *Prevotella copri*, apparently produce succinate rather than propionate as their main fermentation product and succinate accumulation has been reported particularly in the rat gut (De Vadder *et al.,* 2016). The succinate pathway is known to be present in some Ruminococcaceae, such as *Ruminococcus flavefaciens*, which also produces succinate rather than propionate as the end product (Macfarlane & Gibson, 1997). One the other hand, some human colonic bacteria belonging to the Negativicutes class of Firmicutes (eg. *Phascolarctobacterium succinatutens*; Watanabe *et al.*, 2012), have the ability to convert succinate to propionate (Flint *et al.*, 2014; Reichardt *et al.*, 2014). This activity may explain why succinate accumulation is infrequently reported for human faecal samples, although 3 of the 14 overweight human volunteers in one recent dietary study showed elevated faecal succinate concentrations (>30 mM) in samples from a non-starch polysaccharide-supplemented diet (reported in Salonen *et al.,* 2014, Supplementary information). Other Negativicutes bacteria convert lactate to propionate either via the succinate pathway (eg. *Veillonella* spp.) or via the

acrylate pathway (*Megasphaera elsdenii*) (Reichardt *et al.*, 2014) (Fig. 2). The acrylate pathway has also been shown to operate recently in a species of Lachnospiraceae, *Coprococcus catus* (Reichardt *et al.*, 2014).

Formation of propionate and propanol from the deoxy sugars rhamnose and fucose via the propanediol pathway has been demonstrated in dominant gut commensal bacteria belonging to the Lachnospiraceae, including *Roseburia inulinivorans* and *Blautia* species (Scott *et al.*, 2006; Reichardt *et al.*, 2014) (Table 1, Fig. 3). Metabolism of rhamnose and fucose via this pathway has also been reported for *Salmonella* and *Listeria* species (Xue *et al.*, 2008). Other bacteria, including *Bacteroides* species, *Escherichia coli* and *Anaerostipes rhamnosivorans*, are able to degrade deoxy sugars via the propanediol pathway, but produce the pathway intermediate 1,2-propanediol as the final product (Saxena *et al.*, 2010; Rodionova *et al.*, 2013; Bui *et al.*, 2014). 1,2-propanediol can also be produced from other sugars via the glycolysis intermediate dihydroxyacetone-phosphate and methylglyoxal by microbes including *Escherichia coli*, *Clostridium sphenoides* and the yeast *Saccharomyces cerevisiae* (Bennett & San, 2001; Saxena *et al.*, 2010). Methylglyoxal is further metabolised to 1,2-propanediol either via lactaldehyde or via hydroxyacetone (Fig. 3). In *C. sphenoides* it has been shown that 1,2-propanediol formation via dihydroxyacetone-phosphate operates under phosphate limitation and it remains to be established whether it plays a major role in the gut environment. A third pathway for 1,2-propanediol production via lactaldehyde operates from lactate in *Lactobacillus buchneri*. The pathway has been elucidated in a strain isolated from maize silage (Gänzle, 2015), but this species has also been detected in the human gut (Mikelsaar *et al.*, 2016).

E. hallii and *Lactobacillus reuteri*, although unable to grow on fucose or rhamnose, are nevertheless able to utilise 1,2-propanediol to produce propionate and propanol (Gänzle, 2015; Engels *et al.*, 2016) (Fig. 3). Furthermore, metagenomic mining for dehydratases has indicated that further gut anaerobes, including *Flavonifractor plautii*, *Intestinimonas butyriproducens* and *Veillonella* spp. may also be able to produce propionate from this substrate (Engels *et al.*, 2016). Thus, cross-feeding of the intermediate 1,2-propanediol between different bacteria may play an important role in the production of propionate from deoxy sugars. The conversion of 1,2-propanediol to propionate, which is dependent on vitamin B_{12}, takes place in polyhedral bodies, microcompartments that sequester the toxic pathway intermediate

propionaldehyde (Chowdhury *et al.*, 2014). Interestingly, glycerol is converted to 1,3-propanediol and 3-hydroxypropionate in *L. reuteri* and *E. hallii* by the same dehydratase that acts on 1,2-propanediol (Gänzle, 2015; Engels *et al.*, 2016) indicating that glycerol utilization may be the primary function of this enzyme in these species. It is also worth noting that the pathway intermediate 3-hydroxypropionaldehyde, also known as reuterin, is a potent antimicrobial compound (Gänzle, 2015).

Butyrate and propionate formation from proteins and amino acids

Propionate and butyrate are also formed as products from peptide and amino acid fermentation (Fig. 1 & 2), although the numbers of amino acid-fermenting bacteria have been estimated to constitute less than 1% of the large intestinal microbiota (Smith & Macfarlane, 1998; Dai *et al.*, 2011). It is estimated that the colon receives approximately 13 g of protein and peptides per day, and large amounts of soluble protein and peptides were found in intestinal contents of sudden death victims (Smith & Macfarlane, 1998). Peptides seem to be preferred over free amino acids by gut bacteria. Low gut pH and the presence of carbohydrates reduces peptide and amino acid fermentation *in vitro*, which helps to explain why microbial amino acid fermentation is higher in the distal than the proximal colon contents (Smith & Macfarlane, 1998). Amino acid fermentation leads to the production of potentially harmful metabolites (for example phenolic and indolic compounds, amines, ammonia) in addition to branched-chain fatty acids (BCFA) and SCFA (Smith & Macfarlane, 1997; Dai *et al.*, 2011).

In vitro incubations of faecal slurries with individual amino acids showed that propionate was produced mainly from aspartate, alanine, threonine and methionine, whereas butyrate was a major fermentation product from glutamate, lysine, histidine, cysteine, serine and methionine (Smith & Macfarlane, 1997). While several Bacteroidetes have major roles in proteolysis and in propionate formation from peptides (Macfarlane & Macfarlane, 1995), certain Firmicutes species also show high activity on amino acids, notably *Intestinimonas* AF211, which ferments glucose and lysine to butyrate via distinct

pathways (Bui *et al.*, 2015) (Fig. 1). Several different pathways exist for glutamate degradation in butyrate-producing bacteria, which have mainly been studied in *Clostridium* species not originating from gut environments (Barker, 1981; Buckel, 2001). However, there is genomic and metagenomic evidence that they are also present in some gut bacteria (Potrykus *et al.*, 2008; Vital *et al.*, 2014). The glutamate degradation pathways enter the main butyrate pathway either via pyruvate (3-methylasparate pathway; *Clostridium limosum*, *Fusobacterium* spp.) or crotonyl-CoA (4-aminobutyrate pathway, discussed in more detail below, and 2-hydroxyglutarate pathway, found in different Firmicutes including *Acidaminococcus fermentans*, *Clostridium sporosphaeroides*, *Clostridium symbiosum*, *Fusobacterium* spp. and *Peptostreptococcus asaccharolyticus*) (Fig. 1). Some bacteria belonging to the Acidaminococcaceae also degrade glutamate via the 3-methylasparate pathway, but produce propionate rather than butyrate from the intermediate pyruvate (Buckel, 2001) (Fig. 2).

Glutamate degradation to 4-aminobutyrate (gamma-aminobutyrate, GABA) is carried out under acid stress to maintain intracellular pH homeostasis in a number of gut bacteria (Feehily & Karatzas, 2013), and a bacterial isolate exclusively growing on GABA has recently been found (http://www.abstractsonline.com/pp8/#!/4060/presentation/18619). As GABA also acts as a neurotransmitter, the abundance of microbes involved in the production or consumption of GABA may influence mood and behaviour. The pathway for GABA degradation is shared with succinate degradation via succinate semialdehyde and 4-hydroxybutyrate (Fig. 1), and butyrate production from succinate via this pathway has been demonstrated in *Porphyromonas gingivalis* and *Clostridioides difficile* (Ferreyra *et al.*, 2014; Yoshida *et al.*, 2016).

The fermentation routes of other amino acids are less well understood. Histidine is converted to glutamate (Potrykus *et al.*, 2008; Kanehisa *et al.*, 2016), which is in agreement with high levels of butyrate being formed from histidine by faecal microbiota (Smith & Macfarlane, 1997). Alanine, serine and cysteine are broken down to pyruvate (Potrykus *et al.*, 2008; Carbonero *et al.*, 2012), thus product formation depends on the bacterium utilizing those amino acids and their corresponding fermentative pathways. For example, in *Clostridium propionicum*, alanine fermentation leads to the production of propionate via pyruvate, lactate and the acrylate pathway (Buckel, 2001) (Fig. 2). Threonine and methionine are converted

to 2-oxobutyrate, which leads to propionate formation (Fig. 2) (Barker, 1981; Smith & Macfarlane, 1997; Kanehisa *et al.*, 2016). Several routes for the breakdown of asparate exist, via alanine, threonine, oxaloacetate or fumarate (Smith & Macfarlane, 1997; Kanehisa *et al.*, 2016) (Fig. 2), which accounts for the fact that it is mainly converted to propionate in *in vitro* incubations.

Role of CoA-transferases in SCFA metabolism

Propionate and butyrate can be generated from their respective CoA thioesters either by transfer of the CoA-moiety onto another metabolite, or by conversion via propionyl-phosphate or butyryl-phosphate. The second (kinase) route leads to the generation of ATP, but the CoA-transferase route also conserves the energy of the CoA bond in the newly formed CoA-derivative of the co-substrate. Acetate is a common co-substrate in CoA-transferase reactions, and the high acetate concentrations in the large intestine provide a possible explanation for the prevalence of the butyryl-CoA:acetate CoA-transferase route in gut microbes (Louis *et al.*, 2004) (see also section on pH below). Bacteria often carry multipble different CoA-transferases in their genomes, with *Intestinimonas* AF211 encoding at least 14 such enzymes (Bui *et al.*, 2015). It can be difficult to pin-point which gene is responsible for SCFA formation, especially as CoA-transferases tend to have broad substrate specificity. For example, the purified butyryl-CoA:acetate CoA-transferase (*butCoAT* gene product) from *Roseburia hominis* has a similar affinity for butyryl-CoA and propionyl-CoA although the enzyme is clearly responsible for butyrate formation in this species (Charrier *et al.*, 2006) (Table 2). Gene expression evidence in *Intestinimonas* AF211 suggested that the enzyme AtoD-A, responsible for butyryl CoA:acetoacetate CoA-transferase activity, plays a key role in conversion of lysine to butyrate, while the ButCoA gene product mediated the final step in butyrate formation from glucose (Bui *et al.,* 2015). In *Clostridium aminobutyricum*, a CoA-transferase that acts on 4-hydroxybutyrate and butyryl-CoA links the final step of butyrate production to the formation of 4-hydroxybutyryl-CoA further up in the glutamate fermentation pathway (Buckel, 2001). Similarly, *C. propionicum* links the formation of lactoyl-CoA in the acrylate pathway to propionate formation via a CoA-transferase (Buckel, 2001). There are also instances

where different CoA-transferases appear to have evolved for the same enzymatic reaction. Thus, bacteria belonging to the Erysipelotrichaceae do not carry a gene closely related to the butyryl-CoA:acetate CoA-transferase identified in other Firmicutes. Instead, a gene more closely related to propionate CoA-transferases is thought to be responsible for butyrate formation in these organisms (Eeckhaut *et al.*, 2011).

Impact of the gut environment

pH. Gut pH has a major impact on competition between different groups of bacteria within the microbial community. In pH-controlled *in vitro* continuous culture experiments with soluble polysaccharide provided as the main energy source, mildly acidic pH has been shown to curtail the growth of *Bacteroides* spp. relative to Firmicutes and Actinobacteria (Walker *et al.*, 2005; Chung *et al.*, 2016). This is because human colonic *Bacteroides* spp. are generally less able than many dominant Firmicutes to tolerate the presence of short chain fatty acids at pH 5.5 (Duncan *et al.*, 2009). This selective inhibition and the resulting shift in community composition has the consequence of limiting propionate formation and enhancing butyrate production by the community at pH values around 5.5 compared with 6.5-6.8 (Walker *et al.*, 2005; Chung *et al.*, 2016). The impact of pH shifts upon experimentally observed butyrate and propionate concentrations has been successfully modelled mathematically, based on the differing tolerance to low pH of the major bacterial functional groups that comprise the human colonic microbiota (Kettle *et al.*, 2015).

For bacteria that use the butyryl-CoA:acetate CoA-transferase route, acetate consumption and butyrate production are reported to increase at mildly acidic pH compared with near neutral pH (Kettle *et al.*, 2015). Although conversion of glucose to butyrate, 2 CO_2 and 2 H_2 can occur with no net uptake of acetate (Gottschalk, 1979), net acetate uptake is typically observed for species of *Roseburia* and *F. prausnitzii*. Theoretical stoichiometries involving net acetate uptake are shown in Fig. 4A, which also assumes that some of the reducing power that is generated drives proton export, increasing the ATP yield per glucose fermented (Buckel & Thauer, 2013). Incorporation of exogenous acetate via the CoA-transferase reaction results in some loss of ATP production via acetyl-phosphate, but this is more than

compensated by the additional ATP formed from proton export, giving a potential maximum of 4 ATP formed per glucose metabolized when 2 mols of acetate are taken in for each mol of glucose fermented. Interestingly, Fig. 4B shows that the predictions from these stoichiometries (based on the generalised equation shown in Fig. 4A) fit experimental data for the impact of pH on metabolites produced by *F. prausnitzii* and two *Roseburia* spp. in anaerobic batch culture (Kettle *et al.*, 2015). Thus low pH (5.5) tends to increase acetate uptake and butyrate production while near neutral pH (6.7) has the opposite effect. It seems possible that the increased ATP gain associated with net acetate uptake helps to compensate for the effects of low pH and might account for the reliance in the CoA-transferase route for butyrate formation in these bacteria.

Growth requirements. It has been show in a rodent model that limitation of dietary iron intake can dramatically decrease the production of both butyrate and propionate as lactobacilli and Proteobacteria are favoured (Dostal *et al.*, 2012). Populations of *Roseburia*-related butyrate producers appear particularly sensitive to iron availability, while in pure cultures of *R. intestinalis* butyrate production was favoured at high iron concentrations with a switch to lactate production under iron-deficient conditions (Dostal *et al.*, 2015). It remains to be established whether other growth factors also have a major impact on SCFA formation.

Intestinal gases. SCFA formation is also likely to be affected by differences in oxygen concentration in different regions and micro-compartments of the gut due to differences in oxygen sensitivity and metabolic capacity between microbes, as exemplified by the peculiar relationship of *F. prausnitzii* with oxygen (discussed above). Furthermore, the abundance of microbes consuming hydrogen and thereby influencing the hydrogen partial pressure in the gut also influences SCFA formation, as this affects the overall balance of fermentation products formed (Macfarlane & Macfarlane, 2003; Wolf *et al.*, 2016).

Concluding remarks

315 Huge advances have been made in recent years in our understanding of SCFA metabolism in the human
316 gut, and many of the dominant propionate- and butyrate-producing bacteria are available in culture,
317 enabling detailed investigations into their metabolism. Recent work has emphasized that butyrate and
318 propionate can arise from fermentation both of amino acids and of carbohydrates, but the relative
319 contributions of protein and carbohydrate fermentation *in vivo* over the wide range of 'normal' human
320 dietary intakes is not yet clear. We know that high protein, low carbohydrate weight loss diets lead to a
321 disproportionate decrease in butyrate among total faecal SCFA, together with an increased proportion of
322 branched chain fatty acids that are wholly derived from branched chain amino acids and therefore provide
323 an indicator of protein fermentation (Duncan *et al.,* 2007, Russell *et al.,* 2011). This suggests strongly that
324 butyrate production is mainly determined by the supply of non-digestible carbohydrates, rather than by
325 protein fermentation. This may however reflect the particular ecology of butyrate-producing bacteria, as
326 discussed above. In the case of propionate, on the other hand, the major producers of propionate from
327 dietary carbohydrates, the Bacteroidetes, are also important peptide fermenters and the propionate
328 proportion among faecal SCFA was not decreased by such low carbohydrate diets (Duncan *et al.,* 2007). It is
329 also clear that compounds normally regarded as intermediates (eg. succinate, lactate) may accumulate in
330 certain individuals or in particular conditions. This makes it important also to consider the impacts of these
331 metabolites on the host, as for example in the case of succinate which it is suggested may provide health
332 benefits (De Vadder *et al.,* 2016). Lactate is detected as a major fermentation product in breast-fed infants
333 whose microbiota is dominated by *Bifidobacterium* spp. In adults, however, lactate accumulation is
334 associated with dysbiosis, eg, in severe colitis (Hove et al., 1994), that may result in part from a lack of
335 lactate-utilizing bacteria (Belenguer *et al.,* 2007).

336 The ever-increasing availability of genomic and metagenomics sequences is a highly useful resource
337 to foster our understanding of microbial metabolism in the gut, but care has to be taken with assigning
338 function to genes by sequence analysis, which should ideally be complemented by evidence from genetic or
339 enzymatic studies. A renewed interest in isolation and study of gut bacteria (Walker *et al.*, 2014, Browne *et*
340 *al.*, 2016) together with novel systems for gene transfer and knockout on the horizon will enable a
341 thorough understanding of the different members of the microbial community. This will benefit *in vitro* and

in vivo microbial community-based studies to foster our understanding of the different ecological niches of the community members, how they interact with each other and how we can modulate the system by dietary means to optimize SCFA production. The fact that, in general, different phylogenetic groups of bacteria are responsible for butyrate and propionate production suggests that there may be scope for differentially manipulating their production by the gut microbiota.

Acknowledgements

The authors receive financial support from the Scottish Government Rural and Environmental Sciences and Analytical Services (RESAS). We would like to thank Sylvia Duncan for SCFA data of *F. prausnitzii* grown under different pH regimes and for critically reading the manuscript.

The authors declare that they have no conflicts of interest.

References

Allen-Vercoe, E., Daigneault, M., White, A., Panaccione, R., Duncan, S. H., Flint, H. J. et al. (2012) *Anaerostipes hadrus* comb. nov., a dominant species within the human colonic microbiota; reclassification of *Eubacterium hadrum* Moore et al. 1976. *Anaerobe* **18:** 523-529.

Barcenilla, A., Pryde, S. E., Martin, J. C., Duncan, S. H., Stewart, C. S., Henderson, C., & Flint, H. J. (2000) Phylogenetic relationships of butyrate-producing bacteria from the human gut. *Appl Environ Microbiol* **66:** 1654-1661.

Barker, H. A. (1981) Amino acid degradation by anaerobic bacteria. *Annu Rev Biochem* **50:** 23-40.

366 Belenguer, A., Duncan, S. H., Holtrop, G., Anderson, S. E., Lobley, G. E., & Flint, H. J. (2007) Impact of pH on
367 lactate formation and utilization by human fecal microbial communities. *Appl Environ Microbiol* **73:**
368 6526-6533.

369 Bennett, G. N., & San, K. Y. (2001) Microbial formation, biotechnological production and applications of 1,2-
370 propanediol. *Appl Microbiol Biotechnol* **55:** 1-9.

371 Bolognini, D., Tobin, A. B., Milligan, G., & Moss, C. E. (2016) The pharmacology and function of receptors for
372 short-chain fatty acids. *Mol Pharmacol* **89:** 388-398.

373 Bourriaud, C., Robins, R. J., Martin, L., Kozlowski, F., Tenailleau, E., Cherbut, C., & Michel, C. (2005) Lactate
374 is mainly fermented to butyrate by human intestinal microfloras but inter-individual variation is
375 evident. *J Appl Microbiol* **99:** 201-212.

376 Browne, H. P., Forster, S. C., Anonye, B. O., Kumar, N., Neville, B. A., Stares, M. D., Goulding, D., & Lawley, T.
377 D. (2016) Culturing of 'unculturable' human microbiota reveals novel taxa and extensive sporulation.
378 *Nature* **533**: 543-546.

379 Buckel, W. (2001) Unusual enzymes involved in five pathways of glutamate fermentation. *Appl Microbiol*
380 *Biotechnol* **57:** 263-273.

381 Buckel, W., & Thauer, R. K. (2013) Energy conservation via electron bifurcating ferredoxin reduction and
382 proton/Na+ translocating ferredoxin oxidation. *BBA-Bioenergetics* **1827:** 94-113.

383 Bui, T. P. N., de Vos, W. M., & Plugge, C. M. (2014) *Anaerostipes rhamnosivorans* sp. nov., a human
384 intestinal, butyrate-forming bacterium. *Int J Syst Evol Microbiol* **64:** 787-793.

385 Bui, T. P. N., Ritari, J., Boeren, S., De Waard, P., Plugge, C. M., & De Vos, W. M. (2015) Production of
386 butyrate from lysine and the Amadori product fructoselysine by a human gut commensal. *Nat*
387 *Commun* **6:** 10062.

388 Carbonero, F., Benefiel, A. C., Alizadeh-Ghamsari, A. H., & Gaskins, H. R. (2012) Microbial pathways in
389 colonic sulfur metabolism and links with health and disease. *Front Physiol* **3:** 448.

390 Caspari, D., & Macy, J. M. (1983) The role of carbon dioxide in glucose metabolism of *Bacteroides fragilis*.
391 *Arch Microbiol* **135:** 16-24.

392 Charrier, C., Duncan, G. J., Reid, M. D., Rucklidge, G. J., Henderson, D., Young, P. et al. (2006) A novel class
393 of CoA-transferase involved in short-chain fatty acid metabolism in butyrate-producing human colonic
394 bacteria. *Microbiology* **152:** 179-185.

395 Chowdhury, C., Sinha, S., Chun, S., Yeates, T. O., & Bobik, T. A. (2014) Diverse bacterial microcompartment
396 organelles. *Microbiol Mol Biol Rev* **78:** 438-468.

397 Chung, W. S. F., Walker, A. W., Louis, P., Parkhill, J., Vermeiren, J., Bosscher, D. et al. (2016) Modulation of
398 the human gut microbiota by dietary fibres occurs at the species level. *BMC Biol* **14:** 3.

399 Dai, Z. L., Wu, G., & Zhu, W. Y. (2011) Amino acid metabolism in intestinal bacteria: Links between gut
400 ecology and host health. *Front Biosci* **16:** 1768-1786.

401 De Maesschalck, C., Van Immerseel, F., Eeckhaut, V., De Baere, S. D., Cnockaert, M., Croubels, S. et al.
402 (2014) *Faecalicoccus acidiformans* gen. nov., sp. nov., Isolated from the chicken caecum, and
403 reclassification of *Streptococcus pleomorphus* (Barnes et al. 1977), *Eubacterium biforme* (Eggerth
404 1935) and *Eubacterium cylindroides* (Cato et al. 1974) as *Faecalicoccus pleomorphus* comb. nov.,
405 *Holdemanella biformis* gen. nov., Comb. nov. and *Faecalitalea cylindroides* gen. nov., Comb. nov.,
406 respectively, within the family *Erysipelotrichaceae*. *Int J Syst Evol Microbiol* **64:** 3877-3884.

407 De Vadder, F., Kovatcheva-Datchary, P., Zitoun, C., Duchampt, A., Bäckhed, F., & Mithieux, G. (2016)
408 Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis. *Cell*
409 *Metabolism* **24:** 151-157.

410 Dostal, A., Lacroix, C., Bircher, L., Pham, V. T., Follador, R., Zimmermann, M. B., & Chassard, C. (2015) Iron
411 modulates butyrate production by a child gut microbiota in vitro. *mBio* **6:** e01453-15.

412 Dostal, A., Chassard, C., Hilty, F. M., Zimmermann, M. B., Jaeggi, T., Rossi, S., & Lacroix, C. (2012) Iron
413 depletion and repletion with ferrous sulfate or electrolytic iron modifies the composition and
414 metabolic activity of the gut microbiota in rats. *J Nutr* **142:** 271-277.

415 Duncan, S. H., Belenguer, A., Holtrop, G., Johnstone, A. M., Flint, H. J., & Lobley, G. E. (2007) Reduced
416 dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and
417 butyrate-producing bacteria in feces. *Appl Environ Microbiol* **73:** 1073-1078.

418 Duncan, S. H., Louis, P., & Flint, H. J. (2004) Lactate-utilizing bacteria, isolated from human feces, that
419 produce butyrate as a major fermentation product. *Appl Environ Microbiol* **70:** 5810-5817.

420 Duncan, S. H., Louis, P., Thomson, J. M., & Flint, H. J. (2009) The role of pH in determining the species
421 composition of the human colonic microbiota. *Environ Microbiol* **11:** 2112-2122.

422 Duncan, S. H., Hold, G. L., Harmsen, H. J. M., Stewart, C. S., & Flint, H. J. (2002) Growth requirements and
423 fermentation products of *Fusobacterium prausnitzii*, and a proposal to reclassify it as *Faecalibacterium*
424 *prausnitzii* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **52:** 2141-2146.

425 Eeckhaut, V., van Immerseel, F., Croubels, S., de Baere, S., Haesebrouck, F., Ducatelle, R. et al. (2011)
426 Butyrate production in phylogenetically diverse Firmicutes isolated from the chicken caecum.
427 *Microbial Biotechnol* **4:** 503-512.

428 Eeckhaut, V., Machiels, K., Perrier, C., Romero, C., Maes, S., Flahou, B. et al. (2013) *Butyricicoccus*
429 *pullicaecorum* in inflammatory bowel disease. *Gut* **62:** 1745-1752.

430 Engels, C., Ruscheweyh, H. J., Beerenwinkel, N., Lacroix, C., & Schwab, C. (2016) The common gut microbe
431 *Eubacterium hallii* also contributes to intestinal propionate formation. *Front Microbiol* **7:** 713.

Feehily, C., & Karatzas, K. A. G. (2013) Role of glutamate metabolism in bacterial responses towards acid and other stresses. *J Appl Microbiol* **114:** 11-24.

Ferreyra, J. A., Wu, K. J., Hryckowian, A. J., Bouley, D. M., Weimer, B. C., & Sonnenburg, J. L. (2014) Gut microbiota-produced succinate promotes *C. difficile* infection after antibiotic treatment or motility disturbance. *Cell Host Microbe* **16:** 770-777.

Flint, H. J., Duncan, S. H., Scott, K. P., & Louis, P. (2014) Links between diet, gut microbiota composition and gut metabolism. *Proc Nutr Soc* **74:** 13-22.

Gänzle, M. G. (2015) Lactic metabolism revisited: Metabolism of lactic acid bacteria in food fermentations and food spoilage. *Curr Opin Food Sci* **2:** 106-117.

Gottschalk, G. (1979) *Bacterial metabolism.* Springer Verlag, New York, Heidelberg, Berlin.

Hove, H., Nordgaard Andersen, I., & Mortensen, P. B. (1994) Fecal DL-lactate concentration in 100 gastrointestinal patients. *Scand J Gastroenterol* **29:** 255-259.

Kanehisa, M., Sato, Y., Kawashima, M., Furumichi, M., & Tanabe, M. (2016) KEGG as a reference resource for gene and protein annotation. *Nucleic Acids Res* **44:** D457-D462.

Kettle, H., Louis, P., Holtrop, G., Duncan, S. H., & Flint, H. J. (2015) Modelling the emergent dynamics and major metabolites of the human colonic microbiota. *Environ Microbiol* **17:** 1615-1630.

Khan, M. T., Duncan, S. H., Stams, A. J. M., van Dijl, J. M., Flint, H. J., & Harmsen, H. J. M. (2012) The gut anaerobe *Faecalibacterium prausnitzii* uses an extracellular electron shuttle to grow at oxic-anoxic interphases. *ISME J* **6:** 1578-1585.

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016) From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell* **165:** 1332-1345.

453 Lopez-Siles, M., Khan, T. M., Duncan, S. H., Harmsen, H. J. M., Garcia-Gil, L. J., & Flint, H. J. (2012) Cultured
454 representatives of two major phylogroups of human colonic *Faecalibacterium prausnitzii* can utilize
455 pectin, uronic acids, and host-derived substrates for growth. *Appl Environ Microbiol* **78:** 420-428.

456 Louis, P., & Flint, H. J. (2009) Diversity, metabolism and microbial ecology of butyrate-producing bacteria
457 from the human large intestine. *FEMS Microbiol Lett* **294:** 1-8.

458 Louis, P., Hold, G. L., & Flint, H. J. (2014) The gut microbiota, bacterial metabolites and colorectal cancer.
459 *Nat Rev Microbiol* **12:** 661-672.

460 Louis, P., Young, P., Holtrop, G., & Flint, H. J. (2010) Diversity of human colonic butyrate-producing bacteria
461 revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. *Environ Microbiol* **12:** 304-314.

462 Louis, P., Duncan, S. H., McCrae, S. I., Millar, J., Jackson, M. S., & Flint, H. J. (2004) Restricted distribution of
463 the butyrate kinase pathway among butyrate-producing bacteria from the human colon. *J Bacteriol*
464 **186:** 2099-2106.

465 Macfarlane, G. T., & Gibson, G. R. (1997) Carbohydrate fermentation, energy transduction and gas
466 metabolism in the human large intestine. In *Gastrointestinal microbiology vol. I.* R. I. Mackie, & B. A.
467 White (eds). London, Chapman and Hall, pp. 269-318.

468 Macfarlane, S., & Macfarlane, G. T. (2003) Session: Short-chain fatty acids. Regulation of short-chain fatty
469 acid production. *Proc Nutr Soc* **62:** 67-72.

470 Macfarlane, S., & Macfarlane, G. T. (1995) Proteolysis and amino acid fermentation. In *Human colonic*
471 *bacteria - role in nutrition, physiology and pathology.* G. R. Gibson, & G. T. Macfarlane (eds). CRC Press
472 Inc, pp. 75-100.

473 Mikelsaar, M., Sepp, E., Štšepetova, J., Songisepp, E., & Mändar, R. (2016) Biodiversity of intestinal lactic
474 acid bacteria in the healthy population. *Adv Exp Med Biol* [Epublication ahead of print]

475 Morrison, D. J., & Preston, T. (2016) Formation of short chain fatty acids by the gut microbiota and their
476 impact on human metabolism. *Gut Microbes* **7:** 189-200.

477 Morrison, D. J., Mackay, W. G., Edwards, C. A., Preston, T., Dodson, B., & Weaver, L. T. (2006) Butyrate
478 production from oligofructose fermentation by the human faecal flora: What is the contribution of
479 extracellular acetate and lactate? *Br J Nutr* **96:** 570-577.

480 Muñoz-Tamayo, R., Laroche, B., Walter, É., Doré, J., Duncan, S. H., Flint, H. J., & Leclerc, M. (2011) Kinetic
481 modelling of lactate utilization and butyrate production by key human colonic bacterial species. *FEMS*
482 *Microbiol Ecol* **76:** 615-624.

483 Potrykus, J., White, R. L., & Bearne, S. L. (2008) Proteomic investigation of amino acid catabolism in the
484 indigenous gut anaerobe *Fusobacterium varium*. *Proteomics* **8:** 2691-2703.

485 Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C. et al. (2010) A human gut microbial
486 gene catalogue established by metagenomic sequencing. *Nature* **464:** 59-65.

487 Quévrain, E., Maubert, M. A., Michon, C., Chain, F., Marquant, R., Tailhades, J. et al. (2016) Identification of
488 an anti-inflammatory protein from *Faecalibacterium prausnitzii*, a commensal bacterium deficient in
489 Crohn's disease. *Gut* **65:** 415-425.

490 Reichardt, N., Duncan, S. H., Young, P., Belenguer, A., McWilliam Leitch, C., Scott, K. P. et al. (2014)
491 Phylogenetic distribution of three pathways for propionate production within the human gut
492 microbiota. *ISME J* **8:** 1323-1335.

493 Richards, J. L., Yap, Y. A., McLeod, K. H., MacKay, C. R., & Marinõ, E. (2016) Dietary metabolites and the gut
494 microbiota: An alternative approach to control inflammatory and autoimmune diseases. *Clin Transl*
495 *Immunology* **5: e82.**

496 Rodionova, I. A., Li, X., Thiel, V., Stolyar, S., Stanton, K., Fredrickson, J. K. et al. (2013) Comparative genomics
497 and functional analysis of rhamnose catabolic pathways and regulons in bacteria. *Front Microbiol* **4:**
498 407.

499 Russell, W. R., Gratz, S. W., Duncan, S. H., Holtrop, G., Ince, J., Scobbie, L., Duncan, G., Johnstone, A. M.,
500 Lobley, G. E., Wallace, R. J., Duthie, G. G., & Flint, H. J. (2011) High-protein, reduced-carbohydrate
501 weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr*
502 **93:** 1062-1072.

503 Salonen, A., Lahti, L., Salojärvi, J., Holtrop, G., Korpela, K., Duncan, S. H. et al. (2014) Impact of diet and
504 individual variation on intestinal microbiota composition and fermentation products in obese men.
505 *ISME J* **8:** 2218-2230.

506 Saxena, R. K., Anand, P., Saran, S., Isar, J., & Agarwal, L. (2010) Microbial production and applications of 1,2-
507 propanediol. *Indian J Microbiol* **50:** 2-11.

508 Scott, K. P., Martin, J. C., Campbell, G., Mayer, C. D., & Flint, H. J. (2006) Whole-genome transcription
509 profiling reveals genes up-regulated by growth on fucose in the human gut bacterium "*Roseburia*
510 *inulinivorans*". *J Bacteriol* **188:** 4340-4349.

511 Sheridan, P. O., Martin, J. C., Lawley, T. D., Browne, H. P., Harris, H. M. B., Bernalier-Donadille, A. et al.
512 (2016) Polysaccharide utilization loci and nutritional specialization in a dominant group of butyrate-
513 producing human colonic *Firmicutes*. *Microbial Genomics* **2:** 000043.

514 Smith, E. A., & Macfarlane, G. T. (1998) Enumeration of amino acid fermenting bacteria in the human large
515 intestine: Effects of pH and starch on peptide metabolism and dissimilation of amino acids. *FEMS*
516 *Microbiol Ecol* **25:** 355-368.

517 Smith, E. A., & Macfarlane, G. T. (1997) Dissimilatory amino acid metabolism in human colonic bacteria.
518 *Anaerobe* **3:** 327-337.

519 Strobel, H. J. (1992) Vitamin B_{12}-dependent propionate production by the ruminal bacterium Pr*evotella*
520 *ruminicola* 23. *Appl Environ Microbiol* **58:** 2331-2333.

521 Vital, M., Howe, A. C., & Tiedje, J. M. (2014) Revealing the bacterial butyrate synthesis pathways by
522 analyzing (meta)genomic data. *mBio* **5:** e00889.

523 Walker, A. W., Duncan, S. H., Carol McWilliam Leitch, E., Child, M. W., & Flint, H. J. (2005) pH and peptide
524 supply can radically alter bacterial populations and short-chain fatty acid ratios within microbial
525 communities from the human colon. *Appl Environ Microbiol* **71:** 3692-3700.

526 Walker, A. W., Ince, J., Duncan, S. H., Webster, L. M., Holtrop, G., Ze, X. et al. (2011) Dominant and diet-
527 responsive groups of bacteria within the human colonic microbiota. *ISME J* **5:** 220-230.

528 Walker, A. W., Duncan, S. H., Louis, P., & Flint, H. J. (2014) Phylogeny, culturing, and metagenomics of the
529 human gut microbiota. *Trends Microbiol* **22**: 267-274.

530 Watanabe, Y., Nagai, F., & Morotomi, M. (2012) Characterization of *Phascolarctobacterium succinatutens*
531 sp. Nov., an asaccharolytic, succinate-utilizing bacterium from human feces. *Appl Environ Microbiol* **78:**
532 511-518.

533 Weghoff, M. C., Bertsch, J, & Müller, V. (2014) A novel mode of lactate metabolism in strictly anaerobic
534 bacteria. *Environ Microbiol* **17:** 670-677.

535 Wolf, P. G., Biswas, A., Morales, S. E., Greening, C., & Gaskins, H. R. (2016) H_2 metabolism is widespread and
536 diverse among human colonic microbes. *Gut Microbes* **7:** 235-245.

537 Xue, J., Murrieta, C. M., Rule, D. C., & Miller, K. W. (2008) Exogenous or L-rhamnose-derived 1,2-
538 propanediol is metabolized via a *pduD*-dependent pathway in *Listeria innocua*. *Appl Environ Microbiol*
539 **74:** 7073-7079.

Yoshida, Y., Sato, M., Kezuka, Y., Hasegawa, Y., Nagano, K., Takebe, J., & Yoshimura, F. (2016) Acyl-CoA reductase PGN-0723 utilizes succinyl-CoA to generate succinate semialdehyde in a butyrate-producing pathway of *Porphyromonas gingivalis*. *Arch Biochem Biophys* **596:** 138-148.

Zhernakova, A., Kurilshikov, A., Bonder, M. J., Tigchelaar, E. F., Schirmer, M., Vatanen, T. et al. (2016) Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science* **352:** 565-569.

Figure legends

Fig. 1. Microbial pathways for butyrate formation from carbohydrates, organic acids, glutamate and lysine in gut communities. Carbohydrate fermentation to pyruvate via glycolysis is shown in green, butyrate formation from acetyl-CoA in black, amino acid fermentation pathways in blue (intermediates after which the different glutamate pathways are named are highlighted), and lactate and succinate fermentation in purple and pink, respectively. See main text for key enzymes and bacteria harbouring the different pathways. Redox reactions which involve electron carriers are indicated by [H]. CoA-transferase-mediated reactions are indicated by ●. As indicated, co-substrates other than acetate may operate in CoA-transferase reactions in some bacteria (for further detail see main text). CoA, coenzyme A; P, bound phosphate; Pi, inorganic phosphate; PEP, phosphoenolpyruvate; (B_{12}), enzyme dependent on vitamin B_{12}. Dotted line indicates that several intermediate steps are involved.

Fig. 2. Microbial pathways for propionate formation from carbohydrates, organic acids and amino acids. As indicated, amino acids capable of conversion to pyruvate can also give rise to butyrate (Fig. 1). Carbohydrate fermentation to pyruvate via glycolysis is shown in green, propionate formation via the succinate pathway in black, amino acid fermentation pathways in blue, and acrylate pathway for lactate utilisation in purple. See main text for key enzymes and bacteria harbouring the different pathways. Redox reactions which involve electron carriers are indicated by [H]. CoA-transferase-mediated reactions are indicated by ● **(***may be performed by a CoA-transferase or CoA-ligase reaction). Propionate formation

566 from propionyl-CoA in the succinate pathway may involve either a CoA-transferase or phosphate
567 propanoyltransferase/propionate kinase reaction. CoA, coenzyme A; PEP, phosphoenolpyruvate; (B_{12}),
568 dependent on vitamin B_{12},. Dotted lines indicate that several intermediate steps are involved.
569
570 **Fig. 3.** Microbial pathways for propionate formation via 1,2-propanediol. Carriage of the different pathways
571 in gut microbes is indicated by colour. Redox reactions which involve electron carriers are indicated by [H].
572 Propionate formation from propionyl-CoA may involve either a CoA-transferase or phosphate
573 propanoyltransferase/propionate kinase reaction (indicated by a dashed line). Grey hexagon indicates that
574 the reaction is carried out in polyhedral bodies to sequester toxic intermediate propionaldehyde. CoA,
575 coenzyme A; P, bound phosphate; (B_{12}), dependent on vitamin B_{12}. Dotted line indicates that several
576 intermediate steps are involved.
577
578 **Fig. 4.** Butyrate production in bacteria that use the butyryl-CoA:acetate CoA-transferase route. A: General
579 equation for the relationship between acetate consumption and butyrate production, assuming no lactate
580 or formate are produced (modified from Louis & Flint 2009 and Kettle et al. 2015). Etf, electron-transferring
581 flavoprotein; Fd, ferredoxin; P, bound phosphate; Pi, inorganic phosphate. B: Alternative stoichiometries
582 for butyrate production based on A. Experimental data (coloured symbols) refer to *R. intestinalis* L1-82, *R.*
583 *hominis* A2-183 and *F. prausnitzii* A2-165 grown at three different initial pH values (5.5, 6.2, 6.7) (Kettle et
584 al 2015 and Sylvia Duncan, personal communication).
585

586 **Table 1.** Capabilities for butyrate and propionate production among dominant bacterial species detected in

587 faecal samples of human subjects (Qin *et al.*, 2010; Zhernakova *et al.*, 2016)

Phylum (family)	**species**	**Butyrate[1]**	**Propionate[2]**
Bacteroidetes (Bacteroidaceae)	*Bacteroides uniformis*	-	+ (Suc)
	Bacteroides vulgatus	-	+ (Suc)
Bacteroidetes (Prevotellaceae)	*Prevotella copri*	-	+ (Suc)
Bacteroidetes (Rikenellaceae)	*Alistipes putredinis*	-	+ (Suc)
Firmicutes (Lachnospiraceae)	*Eubacterium rectale*	+ (CoAT)	-
	Roseburia inulinivorans	+ (CoAT)	+ (Pdu)
	Roseburia intestinalis	+ (CoAT)	-
	Dorea longicatena	-	-
	Eubacterium hallii	+ (CoAT)	+ (Pdu)
	Anaerostipes hadrus	+ (CoAT)	-
	Ruminococcus torques	-	-
	Coprococcus eutactus	+ (ButK)	-
	Blautia obeum	-	+ (Pdu)
	Dorea formicigenerans	-	-
	Coprococcus catus	+ (CoAT)	+ (Acr)
Firmicutes (Ruminococcaceae)	*Faecalibacterium prausnitzii*	+ (CoAT)	-
	Subdoligranulum variabile	+ (ButK)	-
	Ruminococcus bromii	-	-
	Eubacterium siraeum	-	-
Firmicutes (Veillonellaceae)	*Dialister invisus*	-	+ (Suc)
Firmicutes (Acidaminococcaceae)	*Phascolarctobacterium succinatutens*	-	+ (Suc)
Firmicutes (Erysipelotrichaceae)	*Eubacterium biforme*[3]	+ (CoAT)	-
Actinobacteria (Bifidobacteriaceae)	*Bifidobacterium adolescentis*	-	-

	Bifidobacterium longum	-	-
Actinobacteria (Coriobacteriaceae)	*Collinsella aerofaciens*	-	-
Verrucomicrobia (Verrucomicrobiaceae)	*Akkermansia muciniphila*	-	+ (Suc)

[1]-, absent; +, present; ButK, butyrate kinase route; CoAT, butyryl-CoA:acetate CoA-transferase route.

[2]-, absent; +, present; Acr, acrylate pathway; Pdu, 1,2-propanediol pathway; Suc, succinate pathway (succinate may be the major product formed instead of propionate in some species and/or under some growth conditions).

[3]Reclassified as *Holdemanella biformis* (De Maesschalck *et al.*, 2014). CoA-transferase route is proposed based on closely related butyrate producers within the Erysipelotrichaceae (see also main text, section on CoA-transferases).

598 **Table 2.** Activity of the butyryl-CoA:acetate CoA-transferase of *Roseburia hominis* A2-183 (purified

599 recombinant *butCoAT* gene product expressed in *Escherichia coli* (Charrier *et al.*, 2006)).

600

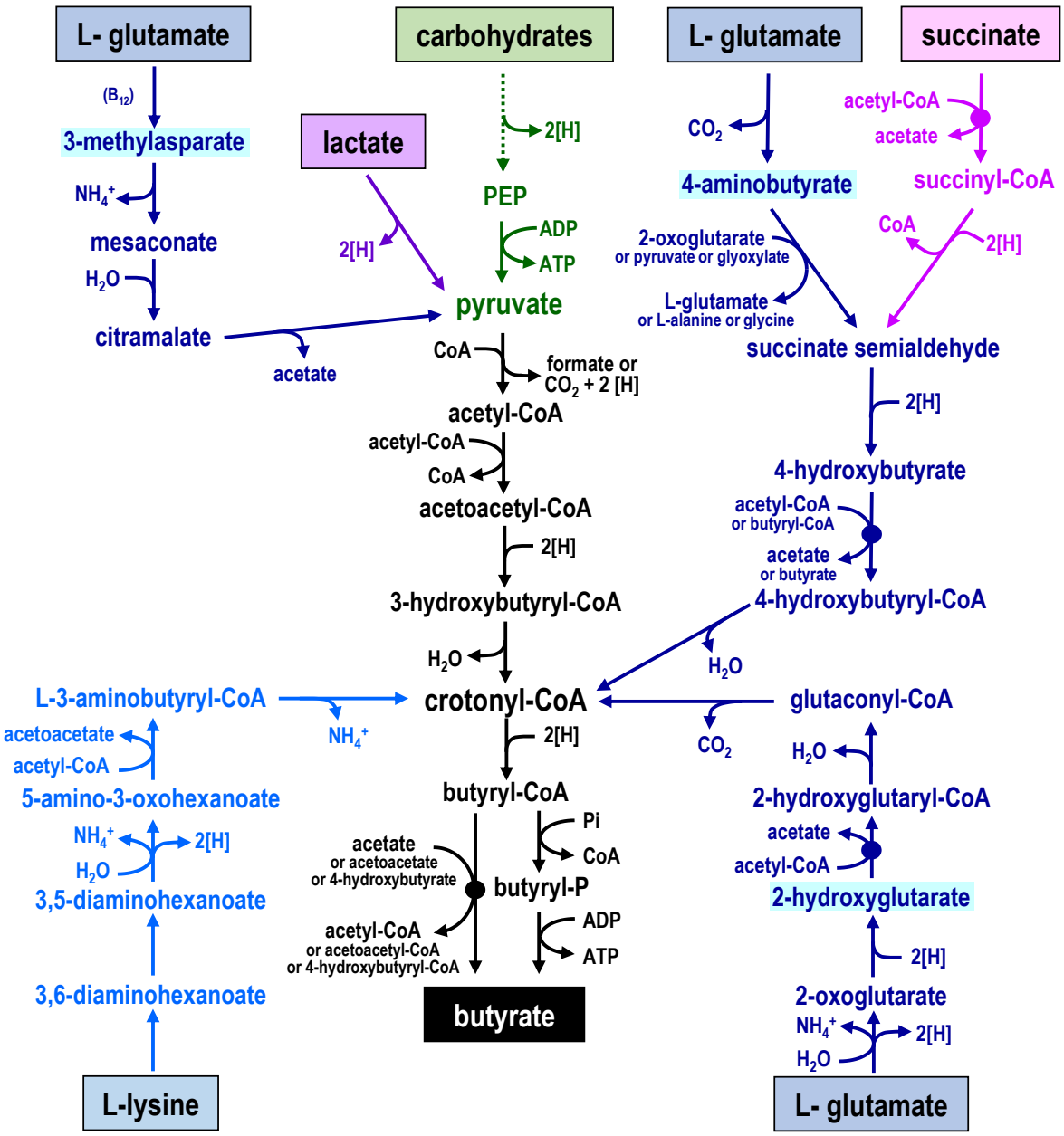
	K_m [mM]	**V_{max} [µmol/min/mg protein]**	**Inhibition by competition with acetate [%][1]**
acetate	6.4		
butyryl CoA	0.098	112	
propionyl CoA	0.099	51	
Butyrate			75
Propionate			70
Isobutyrate			56
Valerate			28

601 [1]No significant inhibition was found for caproate, 3-hydroxybutyrate, 4-hydroxybutyrate, 4-aminobutyrate,

602 lactate, acetoacetate and succinate.

603

Fig. 1


L- glutamate
carbohydrates
L- glutamate
succinate
(B12)
3-methylasparate
NH4+
mesaconate
H2O
citramalate
acetate
lactate
2[H]
2[H]
PEP
ADP
ATP
pyruvate
CoA
formate or
CO2 + 2 [H]
acetyl-CoA
acetyl-CoA
CoA
acetoacetyl-CoA
2[H]
3-hydroxybutyryl-CoA
H2O
crotonyl-CoA
2[H]
butyryl-CoA
Pi
CoA
acetate
or acetoacetate
or 4-hydroxybutyrate
butyryl-P
acetyl-CoA
or acetoacetyl-CoA
or 4-hydroxybutyryl-CoA
ADP
ATP
butyrate
L-3-aminobutyryl-CoA
NH4+
acetoacetate
acetyl-CoA
5-amino-3-oxohexanoate
NH4+
2[H]
H2O
3,5-diaminohexanoate
3,6-diaminohexanoate
L-lysine
CO2
4-aminobutyrate
2-oxoglutarate
or pyruvate or glyoxylate
L-glutamate
or L-alanine or glycine
succinate semialdehyde
2[H]
4-hydroxybutyrate
acetyl-CoA
or butyryl-CoA
acetate
or butyrate
4-hydroxybutyryl-CoA
H2O
glutaconyl-CoA
CO2
H2O
2-hydroxyglutaryl-CoA
acetate
acetyl-CoA
2-hydroxyglutarate
2[H]
2-oxoglutarate
NH4+
2[H]
H2O
L- glutamate
acetyl-CoA
acetate
succinyl-CoA
CoA
2[H]

604

605

Fig. 2

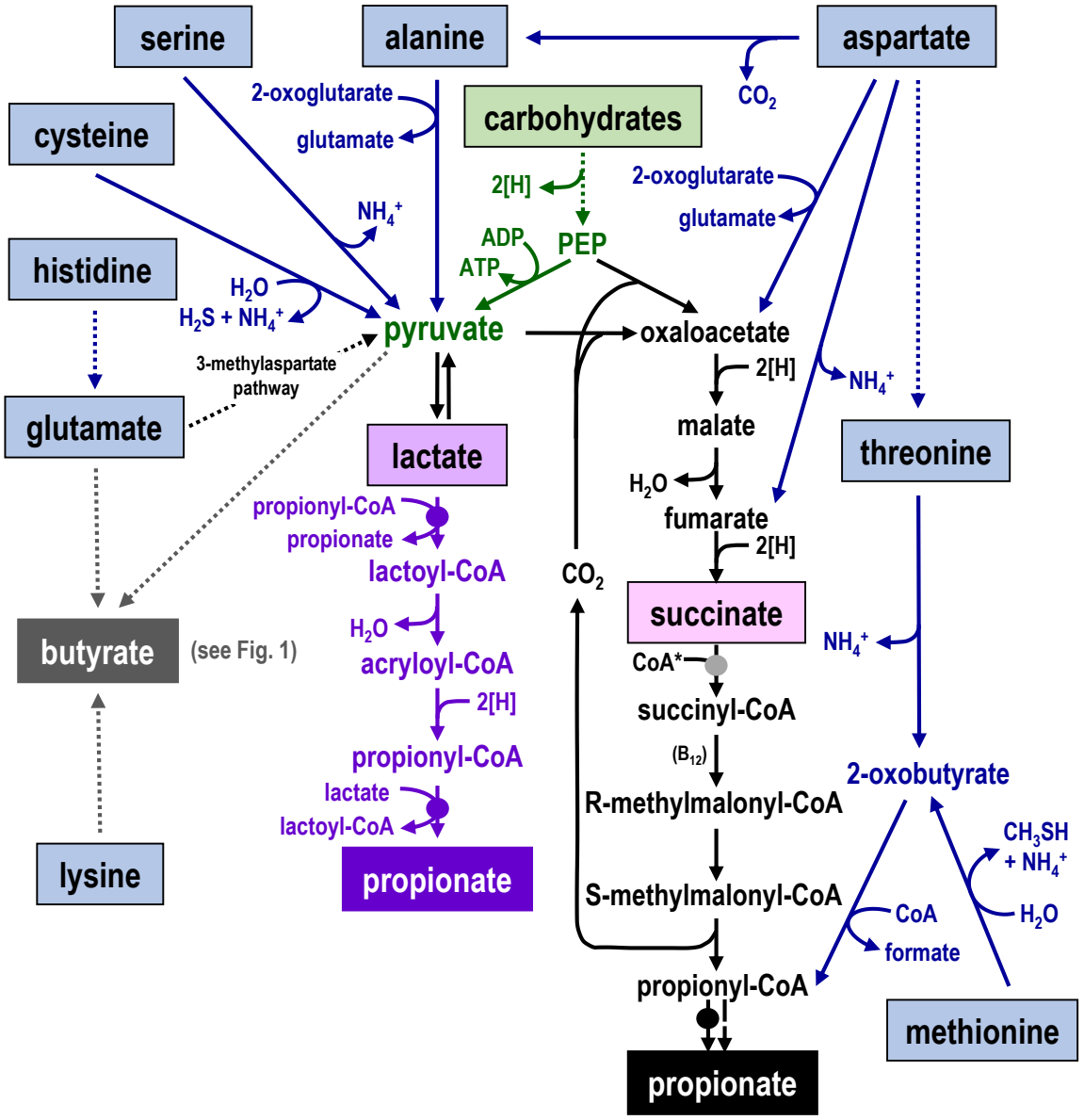
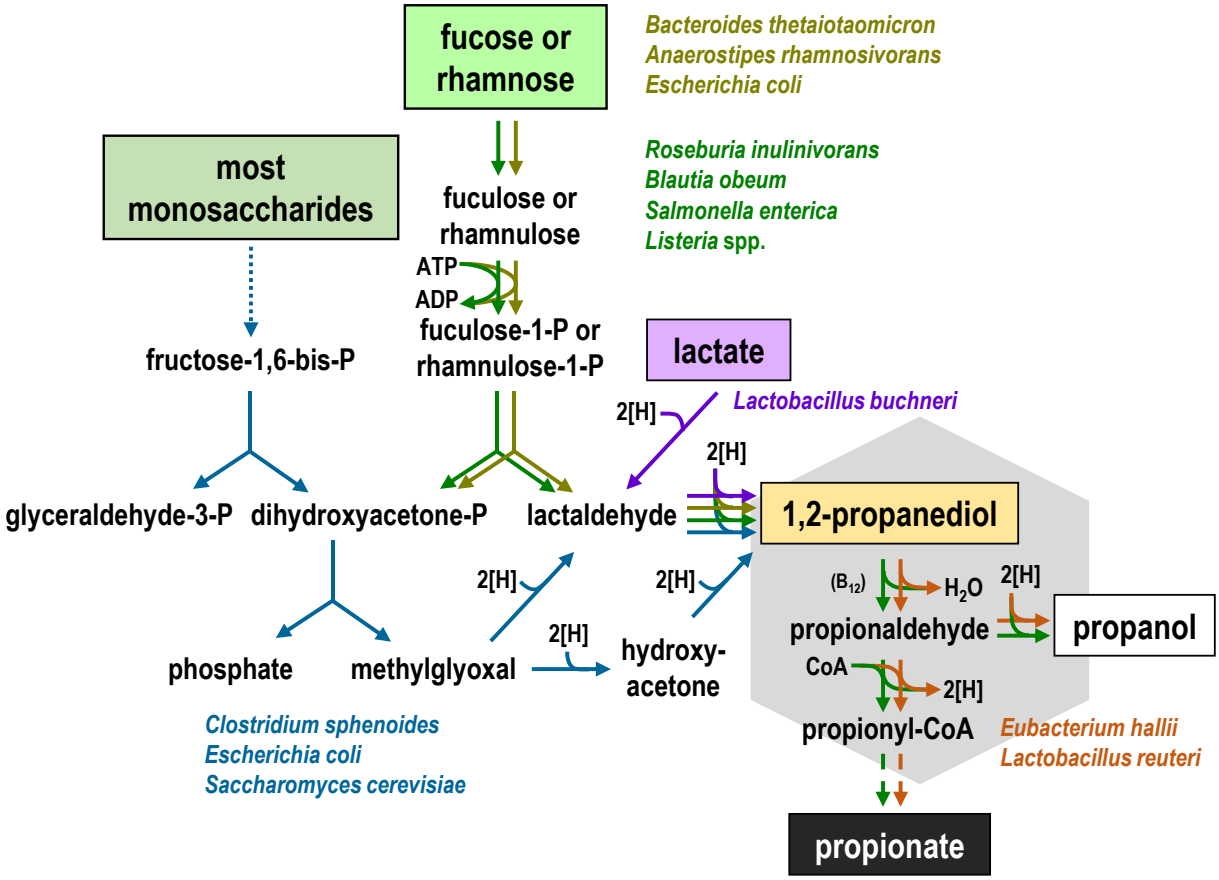

serine
alanine
aspartate
cysteine
carbohydrates
histidine
glutamate
butyrate
(see Fig. 1)
lysine
pyruvate
lactate
propionate
oxaloacetate
malate
fumarate
succinate
succinyl-CoA
R-methylmalonyl-CoA
S-methylmalonyl-CoA
propionyl-CoA
propionate
threonine
2-oxobutyrate
methionine
lactoyl-CoA
acryloyl-CoA
propionyl-CoA
2-oxoglutarate
glutamate
CO_2
2[H]
NH_4^+
ADP
ATP
PEP
H_2O
$H_2S + NH_4^+$
3-methylaspartate pathway
propionyl-CoA
propionate
lactate
lactoyl-CoA
H_2O
2[H]
CoA*
(B_{12})
$CH_3SH + NH_4^+$
H_2O
CoA
formate

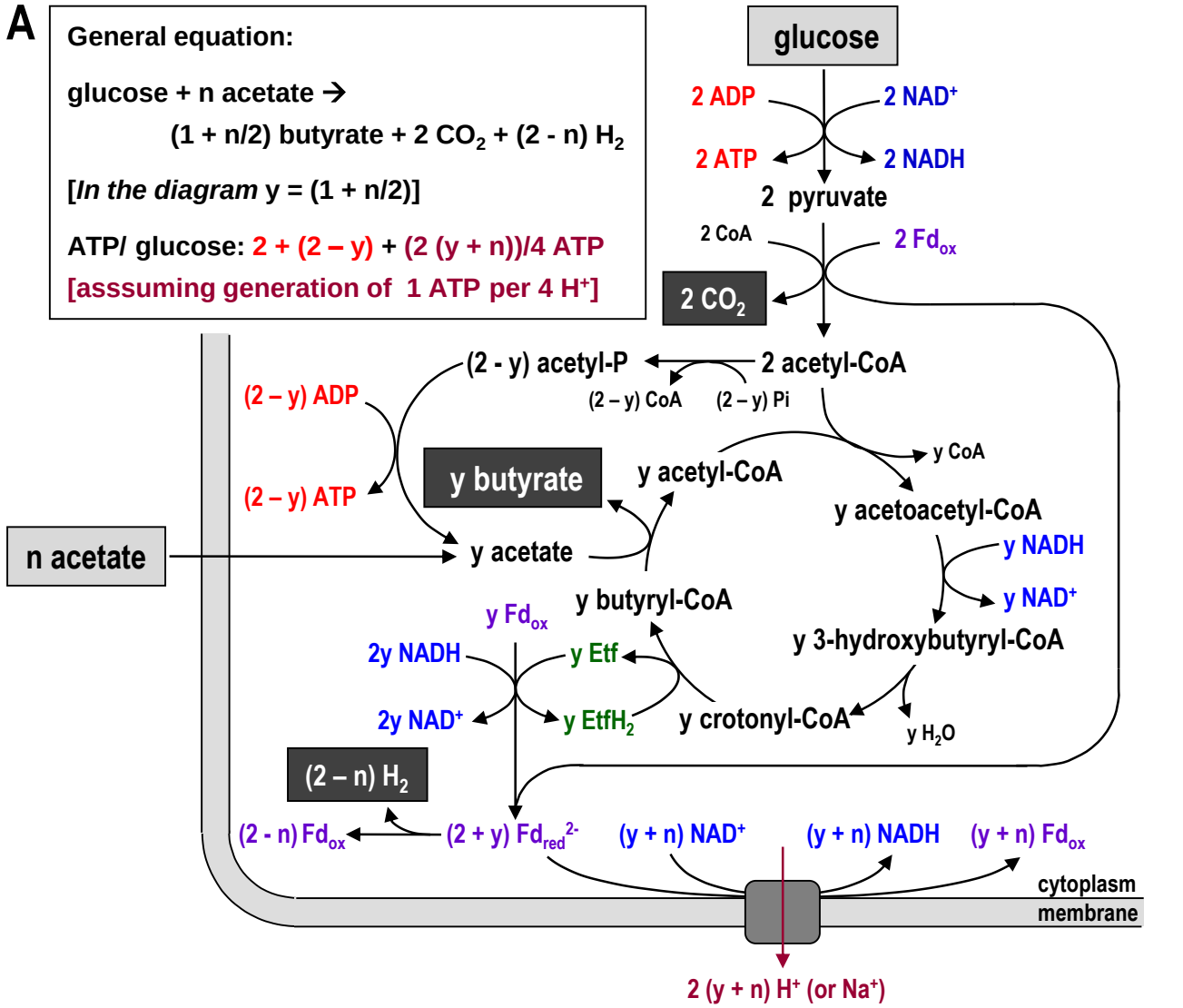
Fig. 3

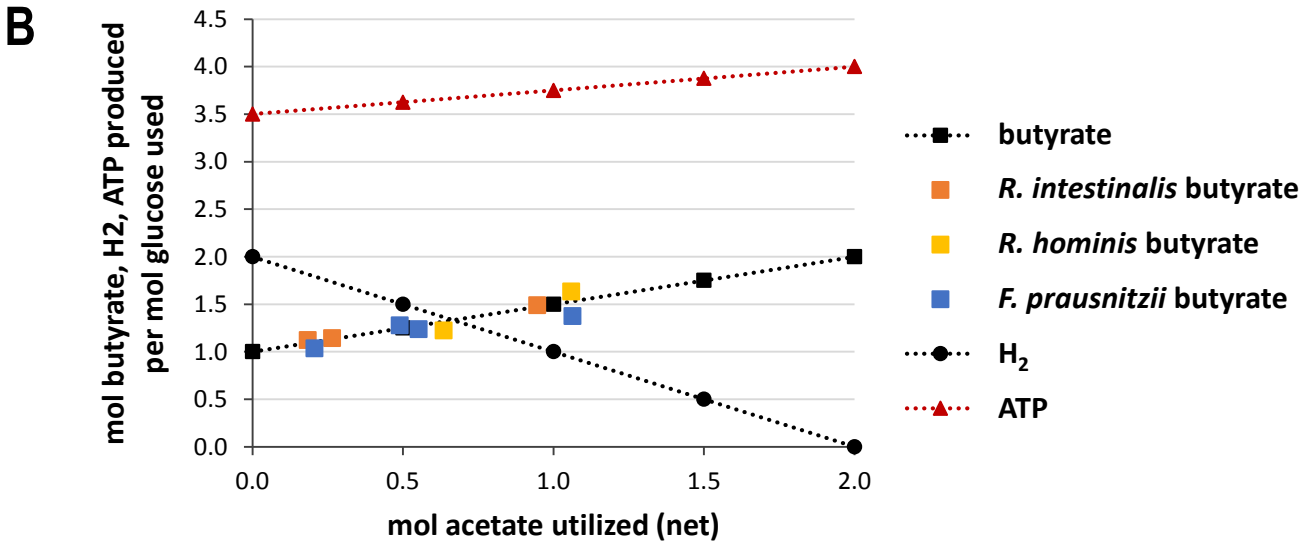

fucose or rhamnose
Bacteroides thetaiotaomicron
Anaerostipes rhamnosivorans
Escherichia coli
most monosaccharides
fuculose or rhamnulose
Roseburia inulinivorans
Blautia obeum
Salmonella enterica
Listeria spp.
ATP
ADP
fuculose-1-P or rhamnulose-1-P
fructose-1,6-bis-P
lactate
2[H]
Lactobacillus buchneri
2[H]
glyceraldehyde-3-P
dihydroxyacetone-P
lactaldehyde
1,2-propanediol
2[H]
2[H]
(B12)
H2O
2[H]
propionaldehyde
propanol
phosphate
methylglyoxal
2[H]
hydroxy-acetone
CoA
2[H]
propionyl-CoA
Clostridium sphenoides
Escherichia coli
Saccharomyces cerevisiae
Eubacterium hallii
Lactobacillus reuteri
propionate

606

607

Fig. 4


A
General equation:
glucose + n acetate →
(1 + n/2) butyrate + 2 CO2 + (2 - n) H2
[In the diagram y = (1 + n/2)]
ATP/ glucose: 2 + (2 – y) + (2 (y + n))/4 ATP
[asssuming generation of 1 ATP per 4 H+]
glucose
2 ADP
2 NAD+
2 ATP
2 NADH
2 pyruvate
2 CoA
2 Fdox
2 CO2
2 acetyl-CoA
(2 - y) acetyl-P
(2 – y) CoA
(2 – y) Pi
(2 – y) ADP
(2 – y) ATP
y butyrate
y acetyl-CoA
y CoA
y acetoacetyl-CoA
n acetate
y acetate
y NADH
y NAD+
y butyryl-CoA
y Fdox
y 3-hydroxybutyryl-CoA
2y NADH
y Etf
2y NAD+
y EtfH2
y crotonyl-CoA
y H2O
(2 – n) H2
(2 - n) Fdox
(2 + y) Fdred2-
(y + n) NAD+
(y + n) NADH
(y + n) Fdox
cytoplasm
membrane
2 (y + n) H+ (or Na+)


B
mol butyrate, H2, ATP produced per mol glucose used
mol acetate utilized (net)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
butyrate
R. intestinalis butyrate
R. hominis butyrate
F. prausnitzii butyrate
H2
ATP